IV.

ON THE FORMATION OF VOLATILE COMPOUNDS OF ARSENIC FROM ARSENICAL WALL PAPERS.

By Charles Robert Sanger.

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In cases of chronic poisoning from arsenical wall papers, the form in which the arsenic leaves the paper has been a long disputed question. Certain well defined symptoms were easily attributable to the local irritant action of arsenical dust in the form of arsenious oxide, the copper greens, etc., and the extension of these symptoms to the digestive organs could be referred to the same cause. Yet, on the other hand, under conditions unfavorable to the separation of arsenical dust, as, for instance, where an arsenical paper underlay one that was perfectly free, cases of poisoning have been very frequent, and to explain them the formation of a gaseous or volatile arsenical compound was assumed.

Experiments to decide this point have been numerous since chronic arsenical poisoning by wall papers was recognized, but none of them until recently have been decisive, as they were with few exceptions carried on under unfavorable conditions and by methods that in point of exactness left much to be desired. Each side has had its adherents, the one asserting that chronic poisoning must be due entirely to dust, the other declaring that the cause lay in the absorption of an arsenical gas, or in the combined action of gas and dust. Many, not believing that the absorption of dust could alone lead to the numerous extreme or obscure cases that have been observed, have used the lack of proof of gas formation as an argument against the possibility of chronic poisoning from arsenical surroundings, although they admitted the local action of dust in certain cases. Further, those who denied the possibility of arsenical poisoning from wall paper under any circumstances considered their position strengthened by the failure to establish definitely the formation of a gaseous compound.

It was in this doubtful state that the question lay at the time when this investigation was begun, some seven years ago. My work was

frequently interrupted and delayed, but about two years ago, I arrived rather reluctantly at results which seemed to show the improbability of the formation of an arsenical gas, though the possibility was as clear as ever. But the extension of bacteriological methods to the investigation has thrown a new light on the matter, and during the past year I have been able to satisfy myself of the correctness of the investigations which have shown that a volatile arsenical compound can be formed by action of certain moulds on organic matter containing arsenic. The nature of the compound is still unknown, and will require extended investigation before it is determined; but it certainly does not seem to be arseniuretted hydrogen, which was the compound commonly supposed to be formed.

Historical.

The danger from arsenical colors in living rooms seems to have been to some extent foreseen before cases of chronic poisoning actually arose, for an order of the Prussian government of January 12, 1815,* directed that the color should be rubbed from green walls when wet, and not when dry.

Gmelin,† in 1839, was the first to call attention to the danger from arsenical wall papers, and considered that the arsenic might be volatilized as some product of the fermentation of the organic matter with which it was mixed.

Riedel,‡ in 1844, says that "air can take up arsenic acid and hydrogen can form arseniuretted hydrogen."

Von Basedow,§ in 1846, considered that the arsenic might be liberated in the form of kakodyl. He gives some cases of poisoning in rooms painted with an arsenical green on a lime ground, and lays stress on the peculiar garlic odor which he notices. This he compares to the odor obtained by boiling arsenious oxide with acetic acid, and says it is similar to that which arises from stuffed birds. The occupants of the room noticed no such odor. Von Basedow notes that the arsenic decreases in the paper by decay, but no analyses are given in support of this assertion.

Krahmer,|| in 1852, was the first to institute experiments to deter-

* Krahmer, Deutsche Klinik, 1852, XLIII 481.

† Ref. by Eulenberg, *Die Lehre v. d. schädl. u. gift. Gasen*, 1865, p. 413, to Karlsruher Zeitung, November, 1839.

‡ Ref. by Krahmer, *loc. cit.*, to Ann. der Staatsarzneikunde, X. 407.

§ Preuss. Med. Zeitung, 1846, X. 43.

|| *Loc. cit.*

mine whether a volatile arsenical compound was liberated. He ridiculed the ideas of Riedel and v. Basedow, and considered that the arsenic could not even leave the walls as dust. His experiments were as follows. Four grams Neuwieder green were mixed with paste and lime taken from a damp part of a ground floor wall, and placed in a two-necked Woulff bottle. After 19 days he observed no odor of garlic, nor indeed could he observe any in the five years that elapsed before his results were published, during which time the mixture remained in the bottle. Five hundred litres of air were then drawn through the bottle for 21 days, the air first passing through alcoholic potassic hydroxide. The solution was then neutralized by hydrochloric acid, and, "on treatment with arsenic free zinc, gave no arsenic." We have here no idea of the delicacy or accuracy of the test used.

Next, a solution of argentic nitrate was substituted for the potassic hydroxide and air drawn through for fifteen days. No change was observed in the silver solution, but it was not tested for arsenic. He then passed the air from the bottle for five days through a hard glass tube heated in two places and obtained no mirror of arsenic. He next thinks that the arsenic compound, if formed, should be mixed with hydrogen before absorption, and so places a hydrogen generator in front of the bottle. The hydrogen generated from 133 grams of zinc, after filtration by cotton wool, was passed through the bottle, then through a solution of alcoholic potassic hydroxide, then silver solution, and finally through the hot tube. He found no trace of arsenic in the solutions or in the tube. The test is not given. During the whole time there was no evidence of mould in the bottle.

Krahmer states that he had lived for eight years in a dry and airy room, the walls of which had received during that time three coats of Neuwieder green, probably five pounds at a time. He never felt any trouble from its presence. He repeatedly examined the dust for *color*, but never found any. To decide the question as to arsenic in the dust, he drew fifty litres of the air of the room through nitric acid, and added to it the dust that had fallen on a piece of paper during that time. The acid was then neutralized with sodic carbonate, evaporated, and the sodic nitrate melted. The residue was dissolved in water, filtered, and the filtrate tested with zinc and hydrochloric acid. He found no arsenic, which is hardly to be wondered at in the presence of so much nitric and nitrous acids. He could not find copper in the filtrate, but this was probably lost by ignition and filtration.

If Krahmer's experiments had been otherwise properly conducted,

the method of examination for arsenic would alone have deprived them of value. Not finding, by his unskilful test, any arsenic in the dust, he concludes at once that arsenic cannot be liberated at all, and uses the result as an argument against the possibility of arsenical poisoning from wall papers.

Arnd,* in 1855, offers the following remarkable explanation: "Arseniates are decomposed by carbon dioxide, setting free arsenious oxide which is volatilized. By evaporation of the water from the paste, arsenical particles are carried off. Sulphide of arsenic on a lime ground is decomposed with evolution of arseniuretted hydrogen, as the lime takes away the sulphur, forming calcic sulphide."

Eulenberg † mentions the following experiment made in 1857 by Halley and Williams, but I can find no account of it or reference to it elsewhere. Several sheets of filter paper were soaked in ammoniacal argentic nitrate and hung up in a closed room of which the paper contained Schweinfurth green. Gas lights were kept burning ten hours, and after they were put out the room was kept closed ten hours longer. The papers, which were blackened, were digested with hydrochloric acid for thirty minutes, and a piece of copper was laid in the solution. A gray deposit was formed on the copper, and the latter, after being washed and dried, was heated in a bulb tube. A white ring was obtained and said to be arsenic, though no further proof was given. Yet, if it were arsenic, which is not decided, it might have had its source in the dust of the room, as well as from a volatile compound.

Halley, in 1858, in a letter to the London Times, ‡ tests the air of a room containing an arsenical wall paper by merely hanging up sheets of paper soaked in ammoniacal argentic nitrate, in which he observes after some time the formation of numerous "well defined crystals of arsenious oxide, visible under a low power microscope." I am inclined to think that this experiment is merely the foregoing, subjected to a newspaper condensation, which has omitted essential details.

Campbell,§ in 1858, made the following experiment. Strips of arsenical paper, about one square foot in all, were placed in a bottle containing a thermometer and fitted with a double bored cork. Through one

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* Verhandl. d. Verein f. Staatsarzneiwissensch. in Berlin, 1855, I. 47.

† *Loc. cit.*, p. 416.

‡ Pharmac. Journ. and Transactions, 1858, p. 428.

§ Ibid., p. 520.

boring passed a right-angled hard glass tube, terminating in a spiral, which could be heated by a broad burner. The other boring held an exit tube passing to a solution of potassic hydroxide. Gas jets were kept burning in the room, and air, at a temperature from 16 to 60°, was drawn from the bottle through the solution. Several trials were made, each lasting one hour, and in no case was arsenic detected. How the arsenic was tested for is not stated, but it is not surprising that none was found. The absurdity of the experiment was shown by Taylor * a week or so later.

Abel,† in the same year, made the following experiments at the instance of the British government. A room was selected having a green unglazed paper containing one fifth of a grain of arsenious oxide per square inch (20 grams per square meter).

1. The room was closed for 36 hours, and then the air was led between four and five hours through a solution of argentic nitrate, and then through a tube containing asbestos soaked in ammoniacal argentic nitrate. No arsenic was found in solution or tube.

2. Five gas flames were burned in the room for several hours, and at the end the air was led through the same reagents with the same negative result. The method used in testing is not given.

3. A glass tube, 3.5 ft. long and 2 in. in diameter, was filled with small pieces of arsenical paper, warmed to 32°, and connected with the reagents as above. The air of the room was led through for a week, and from time to time gas burners were lighted.

4. The air was passed through a strong solution of sulphurous acid and then led for 24 hours through the apparatus.

5. The products of combustion of a gas burner were concentrated by a funnel and led through the apparatus for three days, and again (6) for four days.

7. The paper was roughened by friction and hot air led through for nine days.

8. Strips of arsenical paper with decomposing paste were placed in the same tube, and hot air led over for nine days, with products of gas combustion also. In none of Experiments 3 to 8 was arsenic found in either silver solution or tube. Yet as the method of testing for arsenic is not given, and we do not know its accuracy, the experiments are thus deprived of considerable value.

* Pharmac. Journ. and Transactions, 1858, p. 553.

† Ibid., p. 556; also British Review, October, 1858, and Schmidt's Jahrbuch, 1859, XLV. 20.

9. 600 grains (39 grams) of emerald-green were distributed in a lot of cotton wool and placed in a jar, into which a tube was plunged containing cotton wool and connected with the silver solution. Air was drawn through at 32° for some time, but no arsenic was detected in the solution.

Phillips,* in 1858, repeated the experiment of Halley. Two closets were used, one containing 48 sq. ft. (4.46 sq. m.) of a paper containing 11.8 grains arsenious oxide, as Schweinfurth green, to the square foot (8.3 grams per square meter), and another containing 53 sq. ft. (4.92 sq. m.) of the same paper. In each closet were placed two dishes, one containing a solution of potassic hydroxide and the other ammoniacal argentic nitrate, besides a sheet of paper saturated with the latter. The closets were closed 72 hours and gas was burned 45 hours in one, the temperature being about 25°. No arsenic was found in the solutions by Marsh's test, but the method of preparing the solutions for the test is not given. The paper also contained no arsenic, but was of course dark, and contained crystals of argentic nitrate. Phillips considered that his test proved the absence of the vapor of arsenious oxide, but he has apparently proved the absence of any arsenical dust. The use of the Marsh test is sufficient to account for this, as the amount of dust that might fall in 72 hours from such a paper would quite possibly show no arsenic by the simple Marsh test. As a test for the presence of a gaseous compound like arseniuretted hydrogen, such an experiment is of no value.

Paul,† in the same year, thinks that Campbell's and Abel's work disproves the idea that arsenic is volatilized, and concludes from the negative results that the arsenic cannot leave the paper at all, paying no attention to the possibility of the detachment of arsenical dust.

Oppenheimer,‡ in 1859, was the first to show the presence of arsenic in the dust of a room.

Schmidt and Bretschneider,§ in 1859, placed a mixture of Schweinfurth green, meal, and water in a large flask closed by a double bored cork, through one boring of which a tube led to an argentic nitrate solution protected from the light, the other hole being presumably fitted with a tube reaching under the mixture. In three similar flasks the same mixture was placed, and to them were added, respect-

* Jour. Franklin Inst., [3], XXXVI. 397; also Lond. Civ. Eng. and Arch. Journ., 1858.

† Pharmac. Jour. and Transactions, 1858, p. 616.

‡ Ref. by Eulenberg, *loc. cit.*, to Heidelberger Jahrb. d. Lit., 1859, p. 810.

§ Untersuch. z. Naturlehre d. Mensch. u. Thier. v. Moleschott, VI. 146.

ively, putrid cheese, putrid blood, and yeast, and each flask was connected with an argentic nitrate solution as before. All four flasks were kept at 32° for six weeks, and air from them was passed through the silver solutions. A slight black precipitate was formed in each, but the liquid on being tested in the Marsh apparatus showed no arsenic. No odor was observed, and Schmidt and Bretschneider considered the fermentation to have been stopped. They decide that poisoning cannot take place in damp rooms from a volatile arsenical compound, and that it is more likely that the trouble comes from the detachment of dust in dry rooms. These experiments are more in line with recent work than those preceding, and they are the first that are worthy of much consideration. Certain conditions, not then understood, might have operated against the formation or detection of a gaseous compound. Although the Berzelius-Marsh test may have been used, we do not know the limit in Schmidt and Bretschneider's hands, and the treatment of the silver solution is not given.

Wittstein,* in 1860, advances the idea that the arsenites in the coloring matter are changed to arseniates at the cost of part of their oxygen ($5\ As_2O_3 = 3\ As_2O_5 + As_4$), and that metallic arsenic is volatilized.

Sonnenschein,† in 1869, made the following experiment in a damp room on the ground floor in which the paper was very arsenical, the occupant of which was affected by symptoms attributable to arsenical poisoning. The air of the room after passing through a wash-bottle was led through a hard glass tube heated to redness. After many hours' heating there appeared a perceptible mirror, which Sonnenschein concluded to be arsenic, but he was unable to determine the nature of the volatile compound. The deposit, however, received no confirmatory test.

Fleck,‡ in 1872, conducted the following series of experiments.

1. A five-litre tubulated bell jar resting on a ground-glass plate was covered on the inside with paper smeared with Schweinfurth green (about 15 mgr. arsenious oxide per sq. cm.). The paper was fastened by a paste of potato starch. Through the tubulus passed a cork carrying one right-angled tube reaching to the bottom of the bell jar, and another to just below the cork. The jar was closed and allowed to stand.

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* Quoted by Eulenberg, *loc. cit.*, p. 414; also Schmidt's Jahrb., CX. 88.

† Handbuch d. gericht. Chemie, 1869, p. 153.

‡ Zeitschr. f. Biologie, VIII. 444; also, Dingl. Polyt. Jour., CCVII. 146.

2. A glass flask fitted with tubes like the bell jar was covered on the inside with a paste of gelatine and Schweinfurth green, closed, and allowed to stand.

3. In a bell jar, fitted as in Exp. 1, was placed a dish containing a paste of Schweinfurth green and water.

4. In a similar bell jar was placed a dish containing arsenious oxide and water.

Experiments 1 and 2 should decide whether an arsenical gas could be given off by decomposition of an arsenic compound with organic matter and moisture, while Experiments 3 and 4 would show if any came from an arsenic compound and water alone. In Experiment 1 there was soon a formation of mould and odor of mould, and in Experiment 2 an odor of decaying gelatine. No garlic smell was noticed in either. The temperature was 17 to 18°.

Jar No. 1 and flask No. 2 having been closed for three weeks, at the end of that time the air from the former was led for 24 hours through an absorption bottle containing 150 c. c. water, the air in the jar being renewed about a hundred times. The water was then introduced into a flask containing zinc and acid, from which hydrogen had been passing into argentic nitrate for one hour without result. The silver solution was discolored in 15 minutes, and after an hour was completely black. The filtrate became cloudy on addition of ammonia, but was not tested further for arsenic, the black precipitate being considered sufficient indication of the formation of arseniuretted hydrogen. The same result was obtained from Experiment 2.

Jars 3 and 4 were closed for eight days and the air from them drawn through water as before. The solution from No. 3 gave a very weak action on the argentic nitrate, while that from No. 4 gave none at all.

The first jar and the flask were again closed for some time and the air led through 150 c.c. of argentic nitrate (1–100). The air, before entering the two vessels, passed through a tube of calcic chloride, and between the jar and the argentic nitrate was placed an empty tube to catch dust or anything that might be condensed. No dust or moisture was noticed in this tube. A slight gray deposit was formed in the silver solution and the filtrate gave a turbidity with ammonia.

From the fact that the color of the paper was not lessened, Fleck concludes that the Schweinfurth green was not decomposed, but that the formation of arseniuretted hydrogen was due to decomposition of arsenious oxide. From the amount of silver precipitated he reckons the amount of hydride formed at 0.01 c. c. in the five litres.

Fleck also mixed five grams arsenious oxide with two grams starch

to a paste in a 500 c.c. flask, and noticed formation of mould and crystals of metallic arsenic! Testing this as before, he found arsenic.

No *proof* of the formation of an arsenical gas is given by these experiments. The fermentation, which was greater in the first series of experiments, undoubtedly produced enough volatile organic matter to reduce the silver solution in the light. The failure to test the silver solutions for arsenic deprives the results of value, though it is quite possible, in the light of recent work, that a volatile compound was formed.

Hamberg,* in 1874, tested the air of a room for the presence of a volatile arsenic compound. He used a large dry room which had been papered for 25 to 30 years with a paper of which 1 sq. cm. gave, in the Berzelius-Marsh tube, a thick opaque crust of arsenic 6 cm. long. Arsenic and arsenious acids were found in the coloring matter. The persons who let the room had not perceived any injury to themselves or to others.

A series of tubes was hung on the wall opposite the windows, and the air of the room was drawn through them for a month, the door and windows being closed. During the experiment an alliaceous odor was occasionally observed. The first four tubes were U tubes, of which the first was empty and the next three contained cotton wool. Next came two Liebig bulb tubes with a solution of argentic nitrate in each, and between these and the aspirator was an empty guard U tube. 2,160 litres of air were drawn through the system. After a week a black precipitate appeared in the first silver solution, and later in the second.

The tests were made by the Berzelius-Marsh method. From the first U tube a slight film of arsenic was obtained, in the second (containing cotton) likewise a trace, while the third and fourth contained none. The silver solution was filtered and the precipitate found to be silver, with some sulphide of silver. Ammonia gave a faint yellow precipitate in the filtrate. The latter was precipitated with hydrochloric acid, and the filtrate after evaporation with sulphuric acid introduced into the Marsh flask. In ten minutes a brown film appeared, which increased after an hour to an opaque brown crust. The second silver solution, treated similarly, gave but a faint film.

Hamberg is the first from whose results a definite conclusion can be drawn, and his work is of particular value from having been done

* Pharmac. Journ. and Transactions, [3], V. 81; also, Nord. Med. Archiv, VI. No. 3.

on the air of a room. The methods of the experiment are only open to criticism in that the freedom of reagents and apparatus from arsenic is not stated.

Selmi,* in 1875, investigated the question of the formation of hydrogen by the action of mould. After showing that sulphuretted hydrogen was developed in decaying organic matter over which sulphur had been sprinkled, he reasoned that arseniuretted hydrogen would be formed by the action of moulds on arsenical organic matter. He accordingly sprinkled very finely powdered metallic arsenic over a mixture of horse dung and flour, on which mould was growing vigorously, and placed the preparation in a tubulated bell jar. Strips of paper moistened with argentic nitrate were hung from the top of the jar. The tubulus at the top was closed by a stopper with two holes, carrying tubes for ingress and egress of the air that was drawn through by an aspirator. After five days during which time the bell jar was in the dark, the paper was found to be slightly reddened. It was then treated in a dish with potassic hydroxide, which set free, besides ammonia, a peculiar disagreeable odor. The alkaline residue was then neutralized with nitric acid, evaporated with sulphuric acid to destroy organic matter, and introduced into the Marsh apparatus. After an hour a distinct metallic ring was obtained which, after solution in nitric acid and evaporation, gave a red color with argentic nitrate.

A second trial under the same conditions resulted similarly. In a third and fourth, mouldy lemons were spread with arsenic dust and covered by a funnel from the top of which hung strips of argentic nitrate paper. After 38 hours the organic matter of the paper was destroyed by nitric and sulphuric acids, and the residue, introduced into the Marsh apparatus, gave a well defined ring. Five other trials were made under varying conditions, and in every case a ring of arsenic was obtained. In a tenth experiment arsenious oxide was sprinkled on mouldy starch paste, and placed under a bell jar through

* Ber. d. deutsch. chem. Gesells., VII. 1642 (Corresp.); Schmidt's Jahrbuch, 1875, CLXVIII. 60; Just, Botan. Jahresber., 1876, p. 116. All of these refer to a paper of Selmi's published in the Accademia delle Scienze of Bologna, entitled "Nuovo Processo Generale per la Ricerca delle Sostanze Venefiche," in which, however, the above work does not appear. I have recently obtained a reprint of this article (Bologna, 1875) with which is incorporated "Osservazioni sullo Sviluppo d' Idrogeno Nascente dalle Muffe," and the extract given above is from the latter paper. The abstracters have evidently referred to this separate monograph, and all fail to note the bearing of it upon the question of the formation of a volatile arsenical compound.

which air was drawn. Strips of argentic nitrate paper hung in the bell jar acquired in eight days a red tint, and yielded in the Marsh apparatus a ring of arsenic.

Selmi considered that the results pointed to the formation of an arsine, and that the formation of hydrogen by moulds was confirmed conclusively.

In the light of recent work there may have been a volatile arsenical compound formed in Selmi's experiments. Yet we cannot accept them as conclusive because of the neglect to provide against the possible reduction of the silver paper by the dust particles in the bell jar. Had the filtered air given the above results, they would have been of more value. Besides this the freedom of the reagents from arsenic is not shown. No mention is made of any alliaceous odor from the decomposing matter. The odor from the silver paper after adding alkali may be analogous to that noticed later by Hamberg, Gosio, and myself, but it is not sufficiently characterized by Selmi to draw any definite conclusion in regard to it.

The results of Fleck and Hamberg were accepted by many as conclusive, and more recently the evidence of Selmi has been considered corroborative. It was some time before Selmi's results became generally known. His paper did not obtain wide circulation, and the abstracts quoted above treated his work as bearing only on the general question of the development of hydrogen by moulds, and not on the formation of a volatile arsenical compound.

Professor Chandler of Columbia, in the course of his testimony before a committee of the Massachusetts Legislature in March, 1886, stated that two of his students, Messrs. Morewood and Drummond had, in 1879, under his directions, passed the air from a vessel containing Paris green and paste into an argentic nitrate solution for a number of days, and failed to find any arsenic. The mixture was then allowed to decompose in a warm place and the air was tested for arsenic with the same result. They then covered a square yard of paper with Paris green and paste, placed it in a vessel, and drew air over it into argentic nitrate for many days. The result of this was also negative. As no further details were given, and the work has not, to my knowledge, been published, no conclusion can be drawn as to the possibility of finding small amounts of arsenic by the method used, whatever that might have been.

Bartlett,* in 1880, tried the following experiments. He first

* The Analyst, 1880, p. 81.

passed a stream of hydrogen from sodium amalgam over a quantity of wall paper, free from arsenic and antimony, contained in a large glass vessel, and directed the stream against paper moistened with argentic nitrate and screened from light. There was no action after 12 hours, nor did the addition of ammonia to the hydrogen cause any blackening. He then passed ammoniacal hydrogen from sodium amalgam over moist paper containing a large quantity of arsenic, and obtained what he calls "characteristic reactions" for arsenic, probably a reduction of the silver paper. Of course the blackening of paper cannot be taken as proof of the presence of arseniuretted hydrogen, nor is the formation under these conditions to be expected. Bartlett notes that he intends to pass ammoniacal hydrogen over 20 sq. yd. of paper for a long time in the hope of collecting the resulting argentic arsenite, but he has not, to my knowledge, published anything further on this subject.

Forster,* in 1880, tried the following experiments to ascertain whether arsenious oxide was volatilized from Scheele's green, and whether arseniuretted hydrogen could be given off from a paint containing this pigment. Air was drawn through a bottle filled with glass covered with Scheele's green, and, after filtration by cotton wool, was passed into a solution of potassic hydroxide. One experiment lasted one day, two lasted two days each, and a fourth seven days, the temperature of the bottle being from 45 to 50°. In each case the potassic hydroxide was neutralized by sulphuric acid, and by the Marsh method gave no arsenic. In a second series the bottle was filled with shavings smeared with a paint of linseed oil, turpentine, and Scheele's green. The air was not filtered and passed through two U tubes containing potassic hydroxide and argentic nitrate respectively. Two trials were made for five days and two for six days, at a temperature of 14°; a fifth lasted nine days. The potassic hydroxide solution, treated as before, gave no arsenic. The silver solution was precipitated by hydrochloric acid, and sulphuretted hydrogen led through the filtrate for twelve hours. Not getting a precipitate, Forster concludes the absence of arsenic, but tests further by the Marsh method with a negative result.

Giglioli,† in 1880, after a series of experiments lasting eight months, declared himself opposed to the theory of Selmi, and favored

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* Proceedings Med. Soc. of London, V. 41; Chemical News, 1880.

† Ann. d. R. Scuola sup. d' Agricoltura di Portici, II. 165; Gazz. chim. Ital., 1881, p. 249.

the assumption that chronic arsenical poisoning was due to dust alone. He used moist bread and saccharine liquids, to which arsenious oxide had been added. The preparations were exposed to the air, and, after the mould had formed, were placed in a vessel through which a current of air was passed into an absorbent. Argentic nitrate and auric chloride were used, and in some experiments the air was led through a hot Berzelius-Marsh tube. No arsenic could be detected in the air above the mould, nor was there any odor.

Bischoff,* in 1882, during the examination of some fodder with which arsenic had been mixed with intention of poisoning, placed a part of it, while still moist, in a covered glass dish. After some weeks he observed that mould had collected in the mass, and, on opening the dish, noticed an odor which, from its garlic nature, he concluded to be due to arseniuretted hydrogen. Strips of paper moistened with argentic nitrate were at once turned brown. This was considered by Bischoff to confirm the theory of the formation of arseniuretted hydrogen during fermentation. It is greatly to be regretted that this experiment, which has actually furnished a clue to the recent successful investigations, should not have been carried out properly. There was undoubtedly a volatile arsenic compound present, yet one cannot, in such an important question as this, accept an odor or a blackening of silver paper as indicative of arsenic.

Hamberg,† in 1886, published the result of an investigation, extending over nearly nine years and a half, on the change produced in arsenious oxide in contact with decaying animal matter. I give the experiment in some detail, because an incorrect and misleading idea of it seems to have been obtained from the abstract quoted.

In a twelve-litre flask were placed portions of a body; lungs, liver, kidneys, and intestines, mixed with broken glass and sand. The mixture was moistened with a solution (it is not stated whether it was acid or alkaline) of one gram of arsenious oxide, covered with sand and aluminous earth, and the whole saturated with water. The flask was connected with (1) a tube containing cotton wool, (2) a tube containing test paper, (3) an absorption tube with a 4% solution of argentic nitrate, (4) a U tube to catch any of the silver solution which might be mechanically carried over, and to the last was attached a Finkener aspirator. Air was drawn from outside the laboratory

* Vierteljahr. f. gericht. Med., N. F., XXXVII. 163.

† Pharm. Zeitschr. f. Russland, XXV. 779; Behang t. k. svensk. vetenskabs-akad. Handl., Bd. 12, II., No. 3; Fres. Zeitschr. f. analyt. Chem., XXVI. 788, Ref.

through the flask and system of tubes at the rate of 15–23 litres daily. Mould was noticed in 16 days. From June 4, 1876, to November 21, 1885, the air of the flask was tested at irregular intervals, as detailed below, and the presence of arsenic in the silver solutions determined. In every solution except the first, reduction of the silver was noticed, and very often a yellowish sediment was formed. The odor of the air in the flask was very disagreeable, and in one case brought to mind the odor of either arseniuretted hydrogen or kakodyl. Sometimes the silver solution had a strong odor. Argentic nitrate paper, hung in the flask, turned dark very quickly, but plumbic acetate paper was not affected. Auric chloride paper was introduced in the last year, but was not reduced. Ammonia was given off freely, especially towards the last.

November 21, 1885, as the last silver solution tested gave but a faint deposit in the reduction tube, and an argentic nitrate paper hung in the flask was not blackened, it was concluded that the action had ceased. The residue in the flask was then examined for arsenic. An aqueous extract yielded 149 mgr. arsenious sulphide and the residue from this, extracted with very dilute acetic acid gave 67 mgr. The remainder of the mass, consisting of organic matter, earth, sand, etc., was treated with hydrochloric acid and potassic chlorate, and from the filtered solution 469 mgr. arsenious sulphide were obtained, making 685 mgr. in all, corresponding to 551 mgr. arsenious oxide. The residue from the treatment with hydrochloric acid and potassic chlorate was not examined further, and it is quite possible that the extraction was not complete.

The arsenic was found in the first two extracts as arseniate, but a similar condition could not from the method of extraction be shown in the residue. Undoubtedly the lower oxide was to a great extent changed to the higher. Hamberg concluded that the remaining 449 mgr. had passed away as some gaseous compound, though it is not to my mind certain that these figures are not too large by the amount which may have remained in the last residue.

A consideration of the amount actually absorbed is interesting, and throws some light on the nature of the volatile compound. In the interval between two successive examinations of silver solutions, or when the flask was opened to test odor or action on test paper, much of the gas may have escaped, yet it can be fairly assumed that most of it came in contact with the absorbents. In the first examination (after a run of five months) there were found 1.1 mgr. of arsenious sulphide, corresponding to 0.88 mgr. of the oxide. In the second test

(additional run of three months) the presence of arsenic in sediment and solution was confirmed qualitatively by different reagents, and I assume that the precipitate of arsenious sulphide was not weighable. In the third test (ten months additional run) the sediment gave 0.8 mgr. of sulphide (0.64 mgr. oxide) while the solution gave a " weak reaction." Here again the precipitate seems to have been too small to weigh. In the succeeding determinations the attempt to estimate gravimetrically was abandoned, and the Berzelius-Marsh method was used. Eighteen months had elapsed during which time the action had apparently been at its height, as after sixteen months more the sediment in the fourth test gave no arsenic and the solution an amount much less than the preceding. The rest of the tests gave diminishing amounts from the solutions, while none of the sediments, except the last, contained any arsenic. The silver solutions were precipitated with hydrochloric acid, filtered, and the filtrate, after evaporation with sulphuric acid, was added to the Marsh apparatus.

Unfortunately, the quantitative estimation of arsenic by the Berzelius-Marsh method was unknown to Hamberg, but I have placed the most accurate estimate possible on the mirrors from his description of them, and tabulate the results of the absorption tests on the opposite page.

In the interval between the third and fourth tests, the argentic nitrate solution was one day replaced by a tube containing fused calcic chloride, and the air led through a glowing tube for eight hours. No deposit of arsenic was found. From January (15?), 1882, to March 28, 1882, a second argentic nitrate solution was placed after the first, and, on examination at the same time, contained no arsenic. After this, although a second solution was used, there is no record of its having been tested beyond the statement that the sediment formed was very slight. From July 14, 1885, to October 28, 1885, the argentic nitrate was replaced by a tube containing nitric acid (strength not given), and for most of the time there was an argentic nitrate solution after it. This acid yielded over a gram of ammonic nitrate which was not arsenical. The argentic nitrate solution was kept in a month longer and gave only a faint deposit. This was the last test.

Making allowance for the second test there were found from 2 to 3 mgr. of arsenic as arsenious oxide in the air passed into the argentic nitrate, if we assume that each deposition of an arsenical mirror was carried to completion. This is, however, only from 0.44 to 0.66% of the 449 mgr. which are supposed to have escaped as a gaseous com-

No.	Date of Examination.	Duration of run in Months.	Character of Mirror.	Estimate of Mirror by Standard in Mgr. As_2O_3.*
1	1876, Nov. 10.	5	Estimated from As_2S_3.	0.88
2	1877, Feb. 12.	3	Qualitative determination.	—
3	1877, Dec. 21.	10	Estimated from As_2S_3.	0.64
4	1879, Apr. [15 ?]	16	2 cm. long in 20 min.; "nearly opaque."	0.08
5	1880, May 11.	13	2 cm. long in 35 min.; "slightly transparent."	0.05
6	1880, Sept. [5 ?]	4	2 cm. long; "partly opaque."	0.05
7	1881, Apr. 13.	7	2 cm. long in ten min.; "nearly opaque."	0.06
8	1882, Mar. 28.	11	"Distinct."	0.03
9	1882, May 11.	2	"Weak deposit."	0.02
10	1883, Feb. 9.	9	"Only weak."	0.02
11	1883, Sept. [15 ?]	7	"Only weak."	0.02
12	1885, July 14.	22	"Only weak, transparent."	0.02
13	1885, Oct. 28.	3	From nitric acid; no mirror.	0.00
14	1885, Nov. 21.	1	From solution; "faint, scarcely perceptible."	0.01
			From sediment; ditto.	0.01
			Total	1.89

pound, and from 0.2 to 0.3% of the total amount in the flask. Hamberg states that by addition of all the amounts separated from the silver solutions, partly as sulphide, partly as metallic arsenic, he finds that the greater part of the arsenical gas was not absorbed, and he thinks that possibly the silver solution was only partially able to decompose the gas. Whatever estimate he may have made from the mirrors is not stated; hence we cannot know what amount he thinks was recovered. As my own experiments have shown the inadequacy of 2% argentic nitrate for absorption of the volatile compound and the chance for loss in preparing the solution in the above manner for analysis, the cause of loss is quite clear. The quantitative esti-

* According to the method described by me in these Proceedings, XXVI. 24.

mation of the amount of volatile compound formed in this experiment is subject to too many chances for error to make it of any value except as a means of throwing light on subsequent work. The main fact of the formation of a volatile compound is proved, though the mode of formation is not shown, and its applicability is not extended to the particular question at issue.

Here again has Hamberg given us results which are trustworthy, and his two experiments are the only ones thus far in this historical sketch against which valid objection may not be raised, though in regard to the second I can only repeat what I have said of the first as to absence of detail in the method.

Hamberg, assuming the volatilization to be proved, concludes that in the corpses of persons poisoned by arsenic a similar change takes place, that in the course of years arsenic is given off as a gaseous compound, and that this explains the disappearance of arsenic which has been observed or conjectured by many toxicologists in the examination of parts of exhumed bodies.

Stokes,* in 1888, during an examination of wall papers, fabrics, and domestic articles for arsenic, placed about 300 sq. inches (about 0.19 sq. m.) of arsenical muslin in a glass tube connected with a smaller heated tube. Air was drawn through for six hours, and passed from the hot tube into an argentic nitrate solution. No arsenic was detected in the hot tube or in the solution. The large tube was then heated to 38° and air passed through the system for six hours more, but with the same result. This experiment would only determine whether the arsenical color was itself volatilized, but the duration is so short and the test for arsenic so indefinite that the experiment is of little or no value.

My friend Professor Kinnicutt, of Worcester, Mass., has very kindly allowed me to include here some experiments which he conducted about six years ago but has never published.

1. A room 18 by 20 feet was selected, with a wall paper containing 0.1 grain of arsenic per sq. yd. (7.8 mgr. per sq. m.). The windows and door were closed, and the air of the room was drawn at the rate of about 120 bubbles per minute through a chloride of calcium tube filled with cotton wool and then through 5% argentic nitrate solution. After seven days neither the cotton nor the silver solution contained any arsenic. The latter had a slight black deposit. The method of analysis was essentially the same as in my first series of experiments.

2. A number of pieces of cardboard were covered, by means of

* Chem. News, LVIII. 190.

starch paste, with wall paper containing arsenite of copper, so that in the surface exposed there were about 400 mgr. of arsenious oxide. These cardboards were then placed in a specially constructed tight box with glass windows, arranged so that air could be drawn from end to end. The air entering the box passed through a dilute solution of sodic hydroxide. On leaving it passed through a chloride of calcium tube filled with cotton wool, next through a Liebig bulb tube with 5% argentic nitrate protected from the light, and finally through bulbs filled with nitric acid (sp. gr. 1.12). Air was drawn through the system for two and a half months, averaging seven hours per day. At the end of this time a large amount of mould had formed on the paper. The cotton wool gave a marked test for arsenic, but the silver solution did not show the slightest trace. The nitric acid evaporated with sulphuric acid was also free from arsenic.

3. The box used in No. 2 remained over four years in the cellar of the laboratory, and had become filled with mould, the windows being covered with moisture. A similar series of absorbents was connected, and air was drawn through the system for a week, night and day. The cotton wool, silver solution, and nitric acid were tested as in No. 2, but in no case was any arsenic found.

The evidence presented above, with the exception of the experiments of Fleck and the earlier work of Hamberg, was collected after my first series of experiments was completed. These, which were intended to repeat the work of Fleck and Hamberg, and which were based on the assumption that the volatile compound, if formed, would be arseniuretted hydrogen, are given below. On account of the negative results they are not presented in as much detail as originally intended, for the conclusion derived from them at that time was shown to be erroneous by the results of the second series.

First Series of Experiments.

Fermentation in Solution. — *Experiment* 1. — 20 grams potassic arsenite dissolved in water were mixed with syrup, flour, and part of a yeast cake, and placed in a large flask fitted with a double bored cork. Through one hole reaching to the bottom of the flask, passed a right-angled tube connected with the tubulus of a side-neck test-tube (A) the latter being half filled with a 2% solution of argentic nitrate and fitted with a cork through which passed a right-angled tube to the bottom of the test-tube. In the second hole of the stopper of the

flask was placed an empty Kempff washing bottle (B), reaching just below the stopper and serving as a safeguard against back pressure as well as to catch any of the arsenical mixture that might be mechanically carried up. Through the rubber stopper of the first washing bottle was passed a second (C), containing about 50 c.c. of the same argentic nitrate solution to absorb the volatile compound. This bottle was connected with the right-angled tube of a side-neck test-tube (D), similar in arrangement to A, and serving as a guard against contamination of the silver solution in C from this end. To the tubulus of D was attached the water-pump. The flask was kept at 30° for 36 days and the fermentation was marked, air being drawn through nearly all the time. At the end there was no perceptible change in the solution in the bottle C. This solution was tested as follows. A clean new evaporating dish was heated with about 3 c. c. strong sulphuric acid, and the acid after cooling was diluted with about eight parts of water and introduced into the Marsh reduction flask.* No mirror appeared in 45 minutes, at the end of which time was added the solution obtained from the argentic nitrate. This had been heated to boiling, and precipitated with hydrochloric acid, the filtrate being evaporated with the addition of a few drops of nitric and sulphuric acids until it fumed strongly. Water was then added, and the cold solution put into the reduction flask. No mirror appeared although the action continued for 45 minutes, thus showing no absorption by the silver solution of any volatile compound of arsenic from the fermenting mixture.

Experiment 2. — In the following two experiments the dilution was made very great, in case this condition should affect the formation of the volatile compound. The arrangement of the flask and rear guard tube (A) was as in Exp. 1. Next to the flask was placed a short straight tube filled with fused calcic chloride (B), and to this was connected a washing bottle (C) with 2% argentic nitrate. Diluted syrup solution and yeast were placed in the flask, and to it were added 10 c. c. of a standard solution of arsenious oxide equivalent to 10 mgr. As_2O_3. The apparatus closed at A was left for nine days at the ordinary temperature. Fermentation set in at once and continued until the end. The silver solution was somewhat darkened, probably by the action of light and some volatile organic compound. Air was drawn through the system until the air in the

* The method used in all the experiments was the modification of the Berzelius-Marsh described by me in these Proceedings, XXVI. 24.

flask had been several times replaced. The silver solution, treated as before, gave no arsenic.

Experiment 3. — The arrangement of flask and tubes, contents of flask, etc., were as in Exp. 2, except that a tube containing cotton wool was used instead of calcic chloride. The action went on for 16 days, and at the end of that time there was a slight grayish black precipitate in the silver solution. The latter, however, yielded no arsenic.

Fermentation on Paper. — *Experiment* 4. — A large bell jar, having a tubulus at the top and standing on a well greased ground-glass plate, was fitted with a system of tubes and absorbents similar to those of Exps. 1–3. In the jar were placed several pieces of a wall paper containing 110 mgr. As_2O_3 per sq. m., giving a total amount of 67 mgr. over a surface of about 0.6 sq. m. The back of the paper was smeared with a paste of flour and syrup, to which part of a yeast cake had been added. Air was drawn through the system for fifteen days. There was apparently considerable reduction of the silver solution and a quantity of black precipitate. This was filtered off, and the filtrate treated as previously described. After a 45 minutes' test of the apparatus, the solution gave, after an hour's run, a scarcely perceptible deposit, which did not look like arsenic, and could not be proved to be arsenic.

Experiment 5. — The apparatus was the same as in Exp. 4. Another wall paper was taken, having a surface of about 0.89 sq. m. and containing in itself about 45 mgr. As_2O_3. To the flour paste smeared on the back were added about 7 grams arsenious oxide. Air was passed through for 19 days, and at the end of that time the reduction of the silver solution was more marked than in Exp. 4. The filtered solution was treated as before, and gave, after 30 minutes, a very slight deposit, not resembling arsenic, and giving no confirmatory test.

Experiment 6. — This was a continuation of Exp. 5 with the same apparatus and prepared paper, but instead of the silver solution a small quantity of strong sulphuric acid was used as perhaps a better means of absorbing the volatile arsenical compound. Air was drawn through for 15 days. The acid was darkened and sulphur dioxide had been formed. The acid was evaporated with a little nitric acid, diluted, and added to the reduction flask, but no mirror of arsenic appeared in 30 minutes.

Experiment 7. — This was a continuation of Exp. 5 in exactly the same manner, the sulphuric acid of Exp. 6 being replaced by a silver

solution. Air was passed for eight days, and the reduction was somewhat less than before. The test of the solution was entirely negative.

Experiment 8. — In order to try the effect of alkaline fermentation about 0.5 sq. m. of brown wrapping paper was smeared with flour paste containing 8.5 grams arsenious oxide and a quantity of lime, and placed in the same bell jar without removing the preparation used in Exps. 5–7. The total amount of arsenious oxide was therefore about 15.5 grams. Air was passed for 17 days. There was much mould in the bell jar, and the silver solution was slightly reduced. No arsenic, however, was found in it.

Experiment 9. — In this and in the following experiment the main deviation from the previous trials was in the amount and character of the arsenic compound, and the duration of fermentation. In a large bell jar, fitted with a similar system of absorbents to that of Exp. 1, was placed about 1 sq. m. of a wall paper containing only a small amount of arsenic, and on this was smeared a paste of flour and syrup, to which had been added 10 grams arsenious oxide and part of a yeast cake. After action for 36 days, during which time air was drawn through slowly, the silver solution was examined but gave no arsenic. Very slight reduction had taken place, the light not being as strong as in the previous experiments.

Experiment 10. — The conditions of this experiment were exactly the same as in the preceding, with the substitution of 10 grams of Paris green for the arsenious oxide. The result was also the same.

Direct Experiments on the Air of Rooms. — *Experiment* 11. — The room in which this experiment was performed was a large attic chamber. The surface of paper exposed was about 60–70 sq. m., each containing 280 mgr. arsenious oxide. The frieze and ceiling contained traces only, 0.8 mgr. and 1.0 mgr. respectively, so that the total amount of arsenic may be estimated at about 18 grams. The occupant of the room spent much of his time in it, and showed symptoms attributable to chronic arsenical poisoning. The room was at about 25°.

In order to absorb the volatile compound, if present, a washing bottle similar to those used in the previous experiments was partly filled with a 2% solution of argentic nitrate and connected with an aspirator. In order to free the air from arsenical dust, it passed, before reaching the silver solution, through two tubes filled with cotton wool. In four days, the room being closed, about 750 litres of air were drawn through the bottle. There were then a few black specks in the solution. This, after filtration, was analyzed as before,

the apparatus having been shown to be free from arsenic, and gave no mirror in 40 minutes.

Experiment 12. — The paper in this room had a surface of about 32 sq. m., each containing 30 mgr. arsenious oxide, making a total of about one gram, a rather small amount for the experiment and the size of the room, but the paper was not quantitatively analyzed until afterward. The room was warm, and was kept closed during the trial. No complaint had ever been made by the occupant of symptoms of arsenical poisoning. The apparatus was set up as in Exp. 11, and air was drawn through it rapidly for five days. No reduction of the silver solution was visible. The latter, after treatment in the usual manner, gave no indication of arsenic.

Experiment 13. — This room was considerably smaller than the other two. The total amount of arsenic in it was about 3.6 grams. No symptoms of arsenical poisoning were ever noticed by the occupant, a boy in vigorous health, who occupied the room only at night. The room was closed and kept at 25°. The apparatus was set up as in the previous experiments and 6,720 litres of air were drawn through the system. There was no perceptible darkening of the silver solution, and, on analysis by the same method as in the previous trials, it was found to be free from arsenic.

Experiment 14. — The conditions under which this experiment was performed were, as regards warmth and dampness, more favorable to the formation of a volatile arsenical compound than those of the three previous trials. The occupant of the room suffered from symptoms which were clearly due to arsenic.* The total amount of arsenic present in paper and curtains was about 3.9 grams. During the experiment the room was closed, and the air was at about 30° and damp. The apparatus was similar to that used before, except that between the guard tubes of cotton wool and the silver solution was placed a small quantity of strong sulphuric acid, just as in Exp. 6, as an additional means of absorption. Air was drawn through the system, at intervals for 22 days, the total amount being 1,323 litres. The acid had increased in volume considerably by absorption of moisture, but there was no evidence of reduction as in Exp. 6. The silver solution was slightly darkened and contained a slight black precipitate. Neither sulphuric acid nor silver solution gave any test for arsenic.

In none of the above trials was any alliaceous odor observed, either from the fermenting material or in the air of the rooms.

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* This case (2) is described in the following article.

The result of these fourteen experiments was entirely negative, though they were carried on under widely varying conditions, and by a method better adapted to detect small quantities of arsenic than any before used. On referring to the previous work cited above, the evidence seemed to be greatly in favor of the conclusion reached by my own results, the work of Hamberg being the only positive evidence in favor of the formation of a volatile arsenical compound. Yet there were two points that still gave hope of the correctness of the volatile compound theory: first, the clinical evidence of undoubted poisoning where there was no chance for absorption of dust; and secondly, the fact that in none of the experiments had any quantity of air been tested which approached the amount daily inhaled by an average man.* The amounts used would have perhaps sufficed if the volatile compound were arseniuretted hydrogen, but, supposing it to have been a compound not completely absorbed by argentic nitrate, (as it turns out to be,) a very small amount might have escaped absorption, which as arseniuretted hydrogen would have been easily found.

Though I did not consider the question fully settled, I thought the evidence against the volatile compound theory sufficiently good to warrant the publication of the results thus far obtained. This I was preparing for when notice of the preliminary paper † of Gosio reached me. I wrote to Dr. Gosio at Rome, who after the completion of his work sent me, in September, 1892, copies of the two monographs cited below, and at the same time, with the greatest kindness and consideration, placed at my disposal an admirably prepared set of tube cultures of *Penicillium brevicaule.*

The Work of Gosio.

Gosio's classic monograph ‡ has received little attention from the abstracters, and I therefore present his results here in considerable detail. I judge that much of the work mentioned in my historical sketch has escaped his notice, since he cites Selmi as the most reliable authority for the volatile compound theory, and refers to the very insufficient results of Forster as the basis for support of the dust

* Estimated to be about 12,000 litres in 24 hours, from the statement of Foster, Textbook of Physiology, 5th ed., p. 551, that the amount of tidal air is 500 c.c., and that the number of respirations is 17 per minute.

† Science, XIX. 104, abstract from a preliminary communication to the Congress of Hygiene held in London in 1891.

‡ "Azione di Alcune Muffe sui Composti fissi d' Arsenico," Ministero dell' Interno, Laboratori Scientifici della Direzione di Sanità, Roma, 1892.

theory. He does not mention Hamberg's first paper, and has apparently seen only the wretched abstract of the second quoted above, for he is unaware of some important points in that paper which were similar to his own experience. He quotes the work of Fleck and of Giglioli, and that of Johannson * and Binz † on the tolerance of arsenic by saprophytes. But the most important result he considers to have been obtained by Bischoff, though his judgment is influenced undoubtedly by the fact that his own results have shown the odor to be a positive indication of the presence of the volatile compound, whereas, in Bischoff's experiment it was only an assumption, and not backed by experimental proof. Other investigators than the above are not mentioned.

Gosio at the inception of his work considered that a volatile compound could be formed by the action of mould on arsenical organic matter, though positive proof was wanting, and the mechanism of the reaction was entirely inexplicable. He set himself the following plan of work : —

1. To show whether from arsenical culture ground, exposed to spontaneous inoculation from various surroundings, there could be developed a volatile arsenical compound.

2. If so, to isolate the germs which could effect this transformation and to characterize them.

3. To discover in what arsenical compounds the activity of the organisms manifests itself most markedly; whether this is to be extended to products used in the arts; and to find out what conditions favor and what retard the action.

4. To study the volatile compound.

5. To describe the mechanism by which this gas is produced through the biological energy of micro-organisms.

The first question was answered by an experiment similar to Bischoff's. Potato pulp containing from 0.5 to 1% of arsenious oxide was exposed to the air of a cellar. Abundance of mould appeared in a short time, and at the end of a week an intense alliaceous odor was developed. The pulp was then placed in a glass vessel, through which air was drawn for two weeks into a hot solution of argentic nitrate, the air being filtered by cotton wool. The usual blackening took place, on which Gosio very properly lays little stress, and the solution, freed from silver by hydrochloric acid and filtration, gave "characteristic reactions for arsenic."

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* Archiv f. exper. Pathol., II. 503. † Ibid., XI. 200; XIV. 345.

A similar experiment, in which 120 mgr. arsenic oxide were used, was completed quantitatively, and by the Berzelius-Marsh method a ring of arsenic was obtained weighing 2.8 mgr., corresponding to 4.3 mgr. As_2O_5, or 3.6% of the amount taken. This is unfortunately the only quantitative work in the paper, and I cannot place much reliance on it owing to the inaccuracy of the gravimetric Berzelius-Marsh method with such small amounts.

Pure cultures were now made of some of the moulds developed in the above experiments, and there were isolated *Penicillium glaucum*, *Aspergillum glaucum*, and *Mucor mucedo*. Cultivations were also made of *Bacillus radiciformis*, *B. prodigiosus*, *B. subtilis*, and *Sarcina lutea*. All were then cultivated separately on sterilized arsenical preparations. The odor was noticed only from *A. glaucum* and *Mucor mucedo*. The latter, being a widespread and easily cultivated mould, was selected for further experiments.

Ten Erlenmeyer flasks were fitted with two-holed rubber corks and two right-angled tubes, one passing to the bottom of the flask, the other to just below the cork, each outer end being plugged with cotton wool. Potato pulp containing arsenious oxide and a little tartaric acid was placed in the flasks, which were sterilized, inoculated with a pure culture of mucedo in agar, and connected in series. In the rear was placed a wash bottle of water, in front a 5% solution of argentic nitrate, and a Bunsen pump drew air through the system. The temperature was that of ordinary summer heat. After 28 days the presence of arsenic was established in the filtered solution.

The solution before filtration contained a considerable quantity of a yellow crystalline substance which quickly darkened. This Gosio evidently hoped to connect with the compound $3\,AgNO_3 . AsAg_3$ of Poleck and Thümmel,* overlooking the fact that the latter is formed only in very concentrated solution. On testing the substance no arsenic was found.

The activity of *Mucor mucedo* was further shown by varying the methods of culture, the nutritive soil, and the quality and quantity of the arsenic compounds. Scheele's and Schweinfurth green, in proportions varying from 0.001 to 0.1% of the pulp, gave reactions, but realgar and orpiment gave no odor. Yet, if the action on the sulphides was protracted through many months, there was a slight development. After chemical analysis had confirmed the odor, the latter alone was considered a sufficient indication of arsenic in most

* Archiv d. Pharm., CCXXII. 8.

of the succeeding experiments. To try the action on paper, a tight box was lined with sterilized paper colored by Schweinfurth green and fastened by sterilized starch paste which had been inoculated with mucedo. Air was drawn through the box for 39 days into argentic nitrate which then gave a positive test for arsenic.

The sensibility of the mucedo varies with different conditions: humidity, amount of oxygen present (the formation of gas ceases if air be lacking), and the quantity and quality of the arsenic compounds. The best results were obtained in ground containing 0.01 to 0.05% of substance, while 4 to 5% distinctly retarded the growth. Arsenic acid, arseniates, or alkaline arsenites gave the best results. There can be a tolerance for high doses established, however, if the mould is habituated to a progressive increase. The action goes on better in solid ground than in liquid, and the best nutritive material is a carbohydrate. In albuminoid matter there was but little action, whereas in a mixture of albumen and glucose, it was intense. Whenever the mucedo showed intolerance, other moulds grew which do not decompose arsenical matter, e. g. *P. glaucum.* This explains the failure of other observers (including myself), who used large amounts of arsenic and yet obtained a quantity of mould.

The action of other moulds was examined in the same way, but it was necessary in certain cases to lessen the amount of arsenic. By this means a mould corresponding to the properties of *Aspergillum virens* was found to react. Very slight effects were obtained from *Sterigmatocystis ochracea*, *Cephalothecium roseum*, and *Mucor ramosus.* Finally, from a piece of carrot left in the open air, a new mould was isolated which proved to be identical with the *Penicillium brevicaule* discovered by Saccardo on decaying paper. Experiments on this mould showed it to be capable of more intense action than any other. With milk culture Gosio claims to recognize the odor from 0.02 mgr. of sodic arsenite and thinks it may be still more delicate. In the experiments with *P. brevicaule*, the absorbent was an acid solution of potassic permanganate, but the strength as well as the subsequent treatment is not given. The extreme sensibility of this mould led Gosio to propose its use as a means of testing for arsenic in toxicological work, and the second of his papers is devoted to the working out of this method.

The power of decomposing arsenical organic matter is proposed as a means of distinguishing between two moulds of similar properties.

As "arsenio-bacteria" Gosio specifies those which have a prompt, intense, and lasting action. He thinks that all micro-organisms may

have a slight action in the course of months or years, being led to this conclusion partly by Hamberg's work, though he has no data of his own to prove the theory.

The investigation of the chemical nature of the volatile compound was begun on the assumption that arseniuretted hydrogen was formed, as the silver solution was reduced and arsenic found in the solution. On attempting, however, to precipitate argentic arsenite from solution by ammonia, it was found that the excess of alkali liberated a volatile substance with an intense garlic odor. The following examination was then made. The filtered silver solution was treated with excess of potassic hydroxide in a flask, and the product of the action led over lime and caustic potash to free it from carbon dioxide. The gas then passed over hot cupric oxide and the combustion product, led into baric hydroxide, caused a turbidity. The residue in the cupric oxide tube was digested for two days in cold dilute potassic hydroxide, filtered and washed. The solution was precipitated by sulphuretted hydrogen and the filtrate acidified with hydrochloric acid. The precipitate from the latter oxidized with nitric acid gave reactions for arsenic in the Marsh apparatus. We shall have to take it for granted in the above experiments that the carbon dioxide was all held back by the absorbents, and that the cupric oxide was non-arsenical.

Another filtered silver solution was made alkaline with potassic hydroxide, and air passed from it into platinic chloride for two days. The air from a series of flasks was also passed into this absorbent for ten days. In both cases no satisfactory results were obtained.

In the solution after treatment with alkali, arsenic was looked for, but only faint traces were found. This would be against the formation of arseniuretted hydrogen, yet Gosio inclines to the belief that there may be traces of it formed.

Considerable space is given to discussing the mechanism of the reaction, yet, in view of the fact that the compound has not been isolated, such discussion is purely speculative. Gosio refers to the work of Pollaci,* Selmi,† and Fitz and Mayer ‡ on the formation of hydrogen by action of lower organisms, and quotes the statement of Nencki § that bacteria may decompose water into H and OH, which would cause a hydrogenization and a hydroxylization, a double action

* Reference not given by Gosio.

† Presumably the same reference as given by me above.

‡ Ber. d. deutsch. chem. Gesells., XI. 1880; XII. 474.

§ Ibid., XII. 474.

which he evidently thinks may take place in this case, giving rise to arseniuretted hydrogen and an arsenical compound of carbon with the hydroxyl group. On the other hand, he refers to the work of Missaghi,* which opposes the formation of hydrogen by moulds.

The second paper† of Gosio need be referred to very briefly. The action of *P. brevicaule* proved so sensitive as to suggest a very delicate method for the detection of arsenic in presence of large amounts of organic matter. The method is carried out as follows. A test-tube is constricted about 20-30 mm. from its lower end, and the bulb thus formed filled with moist cotton wool. A strip of paper is cut in halves and the suspected substance placed between the slices, which are then put into the test-tube and sterilized. Inoculation is then made, and in a day or two the characteristic odor is developed. The temperature is best about 37°. Should further proof be desired, a rubber stopper with right-angled tubes may be inserted in the test-tube, and air drawn slowly through it into a sulphuric acid solution of potassic permanganate kept at 60 to 70°. This solution after proper treatment is introduced into the Marsh flask.

Undoubtedly in cases where the amount of arsenic is very small, and in contact with a large amount of organic matter, this method would be excellent, but I cannot see that in general medico-legal work it has any advantage over the common methods, particularly as it requires much time and cannot be made quantitative. It is simply a very interesting micro-biological method. The exceedingly great delicacy claimed for it (1 part of sodic arsenite in 1,000,000 being detected in a milk culture) is not surprising when we consider the amount of substance that can be recognized by the sense of smell, E. Fischer and Penzoldt,‡ for instance, claiming to have detected one four hundred and sixty millionth of a milligram of mercaptan.

The correctness of Gosio's work is unquestionable, and to him is due the credit of settling this much vexed question. Yet it seemed to me that the importance of the matter demanded a substantiation of his results by repetition of his work, particularly as so many observers beside myself had obtained opposite results, and would be better satisfied to know that the action of the moulds had been confirmed by another investigator in another country. I have therefore made some experiments similar both to those of my first series and to those

* Gazz. chim. Ital., V. 419.

† "Sul Riconoscimento dell' Arsenico per Mezzo di alcune Muffe," Roma, 1892.

‡ Ann d. Phys. u. d. Chem., CCXXXIX. 131.

of Gosio, using the material from the culture tubes sent me by him, which were five in number and contained *P. brevicaule* in potato strips to which had been added different preparations of arsenic.

SECOND SERIES OF EXPERIMENTS, USING P. BREVICAULE.

Experiment 15. — One gram arsenious oxide was mixed in a litre flask with flour and water which, without sterilization, was inoculated from the tube marked "patata esente di arsenico." A similar system of tubes to that described in Exp. 3 was attached to this flask, including the usual absorption bottles filled with 2% argentic nitrate, and air was drawn through the system for about two weeks, during which time the formation of mould was abundant and fermentation marked. The reduction of the silver solution was slight. On opening the flask no odor was noticed except that characteristic of mould. Although there was little evidence of the formation of a volatile compound, the silver solution was tested in a similar manner to that described in Exp. 1, but with entirely negative result.

Experiment 16. — One gram arsenious oxide was mixed with unsterilized flour paste, and, after inoculation with the same preparation as in Exp. 15, smeared on a large sheet of filter paper. This was placed in a bell jar over a ground-glass plate, and the jar fitted with a system of tubes and absorbents similar to that described in Exp. 4. Air was drawn through for about two weeks. The formation of mould was abundant, but no alliaceous odor was observed. The silver solution was but slightly reduced and gave no test for arsenic.

In these two experiments, the *P. brevicaule* could not tolerate the amount of arsenic under the conditions as well as the common moulds, hence the latter flourished to the entire exclusion of the former. This was shown by Mr. Roscoe Pound, of Lincoln, Nebraska, who very kindly examined the sheet in Exp. 16 and found the only Penicillium present to be *P. crustaceum.*

The conditions of Exps. 15 and 16 are not unlike those of the previous negative experiments.

Experiment 17 *a.* — In this the method of Gosio was quite closely followed. Three 250 c. c. Erlenmeyer flasks were fitted with rubber stoppers through which passed two right-angled tubes, one reaching nearly to the bottom of the flask, the other to just below the cork. The outer end of each tube was plugged with cotton wool. Sufficient potato pulp was placed in each flask to make a layer of about half an inch, and it was moistened with a solution of sodic arseniate containing about 0.5 gram to 100 c. c. Each flask contained about 100 mgr.

As_2O_5. They were then heated for two hours in a steam sterilizer and allowed to stand 24 hours. This was twice repeated. Each flask was then inoculated with a sterilized platinum point from the tube marked "patata bagnata in una soluzione di As_2O_5." The three flasks were then connected together and in the rear was joined a side-neck test-tube containing 2% argentic nitrate. In front was placed an additional layer of cotton wool in a chloride of calcium tube, then two silver solutions of the same strength as the first. The joints of the apparatus were wired and were tight. A current of air was drawn very slowly through the system day and night, the amount being measured. In one day the growth at the points of inoculation began, and in three days there was a patch half an inch in diameter around each spot. The silver solution had darkened slightly. The temperature during the trial was about 25°.

At the end of twelve days, as it became necessary to change the place of experiment, the flasks were disconnected and packed for transportation. Up to this time no difference could be noted between this experiment and the many preceding, except that the mould was in smaller quantity and apparently more homogeneous. The first silver solution was somewhat reduced, there being a slight black deposit, but the second was not changed. But as soon as the flasks were disconnected (they were not uncorked) an alliaceous odor could plainly be perceived at the tubes, *a point that I had never observed in any previous experiment.* The silver solutions were then tested, the method being for certain special reasons slightly modified. The unfiltered solution was precipitated by a very slight excess of sodic chloride, and the filtrate evaporated with sulphuric acid until it fumed strongly. The diluted residue was boiled with a very little sodic sulphite, and the excess of sulphur dioxide expelled. The cooled solution was then introduced into the Marsh flask which had been running one hour without sign of a mirror in the deposition tube. Here may it be said that all reagents used had been subjected to the most rigid test, the stream of hydrogen from the generator not giving any arsenic whatever in a seven-hour run. Twenty-five minutes after introduction of the prepared solution a clearly defined mirror of arsenic was deposited *which was the first that I had obtained in my experiments on this subject.* The amount was small, about 0.01 mgr., but, taken in connection with the odor, it was very satisfactory considering the small amount of mould and the duration of its action. One hundred and five litres of air had been drawn through. The second silver solution treated in precisely the same way gave no mirror.

b. Three weeks elapsed before the experiment could be continued, during which time the flasks remained sealed by the cotton wool. In two of them the growth did not seem to have increased much, but in the third the ground was completely filled with mould, which did not seem, however, to be entirely homogeneous. I am not sure that the stopper of this flask may not have been slightly loosened. The odor of garlic was very strong.

The flasks were connected with silver solutions as before, and air led through each day for 17 days. In four days there appeared on the lower end of the entrance tube of the first silver solution a dark mirror, which increased slightly and was *apparently* the only deposit formed. The temperature during the first week was about 20°, but afterwards about 25°. At a very rough estimate 170 litres of air were passed through the system.

The first silver solution was poured out of the bottle, and the latter merely rinsed with water. The solution was heated nearly to boiling, and excess of hydrochloric acid was added. At this point I was struck by the strong odor coming from the warm mixture, it being noticeable at a distance of two feet from the beaker. It strongly resembled the garlic odor of arsenic, and also recalled the odor from a solution of iron in dilute acid. My assistant, in making the previous precipitation with sodic chloride, noticed no odor. The odor grew weaker as the mixture was kept warm, but the argentic chloride was filtered before it had entirely disappeared. The filtrate, after addition of a little more nitric acid, was evaporated with sulphuric acid to fuming, diluted, and added to the Marsh flask which had been running for forty minutes without a trace of arsenic. In ten minutes a mirror began to appear, was very heavy in thirty minutes, and at its maximum in fifty. It was clear and well defined, but too heavy for accurate estimation. I placed it at 0.07 mgr., which is a low estimate.

Besides the mirror of silver (?) on the end of the tube in the absorption bottle, there was evident on closer inspection a small amount of a nearly colorless (perhaps slightly yellow) gelatinous-looking substance adhering to the bottom and walls of the bottle. This dissolved easily in the few drops of strong nitric acid added to dislodge the heavier black deposit, though the action of the acid upon it was masked by the nitrous fumes from the solution of the mirror. The solution was precipitated with hydrochloric acid, evaporated to fuming with sulphuric acid, diluted, and added to a Marsh flask which had been running for 30 minutes without evidence of arsenic. A mirror

appeared in 30 minutes which was at its maximum in 60. The amount formed was 0.025 mgr., making 0.095, or probably 0.1 mgr. in all, from this solution.

It was possible that the first silver solution had not absorbed all the volatile compound, as was conjectured in Hamberg's case. Bearing in mind the probable volatilization of arsenic in the method employed in the first solution, the second, which contained little or no deposit, was evaporated with considerable nitric acid to incipient fusion, during which no alliaceous odor was noticed. The residue was taken up with dilute nitric acid, precipitated with hydrochloric acid, and the filtrate, after evaporation with sulphuric acid and dilution, added to a Marsh flask in which the absence of arsenic had been shown by a 40-minute run. After an hour, the mirror, which appeared slowly, was at its maximum, and was estimated at 0.015 mgr. This shows conclusively that argentic nitrate in such dilution does not absorb the volatile compound with any degree of completeness.

To prove that no arsenic could have entered from the air of the room, the rear solution of silver was treated in the same manner, and gave no trace of arsenic.

The total amount of arsenic (as As_2O_3) obtained from this series of flasks was 0.12 mgr. No further satisfactory quantitative result can be adduced for many reasons. During the interval between Exps. 17 *a* and 17 *b*, much of the compound may have escaped; the silver solutions did not absorb all of the compound, and the method of treating the first solution at least was incorrect, being based on the old assumption that arseniuretted hydrogen was the product. Hence 0.12 mgr. does not represent by any means what may have been formed. Yet if one reckons 300 mgr. of arsenic (as As_2O_3) to the flasks, the amount recovered is 0.04% of this, a proportion which for all we know may be 100 times too small.

The flasks were now uncorked. The odor, though fainter, was distinctly perceptible and was confirmed by others in the laboratory. Dr. W. G. Farlow has had the kindness to examine the growth for me, and reports that the first two flasks contained only *P. brevicaule* "fruiting and in good condition," while the third, which I suspected was not homogeneous, contained also *P. glaucum*, though the amount "as compared with the *P. brevicaule* is less than one would suppose on looking at the flask without examining microscopically."

Though the above experiment confirmed the results of Gosio to my complete satisfaction, the following trial is of great interest on account of the small amount of arsenic and its source.

Experiment 18. — Three 200 c.c. Erlenmeyer flasks were fitted as in Exp. 17, with rubber stoppers and tubes, the latter plugged with cotton wool. A wall paper was selected having 115 mgr. arsenious oxide per square meter, the color, a dark red, suggesting an aniline compound mordanted with an arseniate. 3 square decimeters of this were cut into strips and placed between several slices of potato, which were put into each flask. The total amount of arsenic used was 3.45 mgr., reckoned as As_2O_3. The flasks, after addition of a little water, were sterilized for two and a half hours at 100–105°, and showed no evidence of mould for six days afterward. The potato, which was still moist and impregnated with the red coloring matter, was then inoculated with the culture in the tube marked "patata contenente una striscia di tappezzeria arsenicale," and the flasks were connected with a series of absorbents similar to that of Exp. 17. The current of air was not drawn through the system until the flasks had stood for a week, the temperature being from 20 to 25°. No mould appeared for four days, and the growth was then very slow, was confined to the first two flasks, and the amount was very small. Indeed, after 17 days had passed without much apparent action, the silver solution being but slightly affected, I disconnected the flasks, not intending to pursue the experiment further. Yet on opening the flasks the alliaceous odor though faint, was perfectly plain, and I proceeded to test the first silver solution. This was poured out, and the very slight deposit removed with a little nitric acid was added to the solution. The latter was evaporated with nitric acid nearly to fusion, taken up with dilute nitric acid, desilverized, and prepared as usual for the Marsh flask. This had been in action 40 minutes, and was free from arsenic. At the end of an hour after introducing the solution there was a small but perfectly plain mirror, which I estimated at 0.005 mgr. The second silver solution was not examined.

Dr. Farlow kindly examined the first flask of the series, and found "*P. brevicaule* in good condition but not pure, for there was another species of Penicillium present not in very good fruit. The second species may have been a small form of *P. glaucum*, but I do not think I can say certainly from the material examined, which was too young."

The amount of the volatile compound determined in this experiment is 0.14% of the total present. This estimate is more accurate than in Exp. 17, and the chief error is in the absorbent, though the amount is only a part of what might have been formed in the course of time.

The reagents used in Exps. 17 *b* and 18, being in another laboratory, were subjected to the same rigid tests, and the absence of arsenic was proved. The dishes and utensils were proved free by blank tests.

Discussion.

The scope of this paper has gone far beyond my original intention, which, as stated above, was simply to repeat the work of Fleck and of Hamberg. As the important results of Hamberg and of Gosio have not been given the publicity due to them, not to speak of the comparative obscurity into which the work of many of the other investigators has fallen, I feel that the somewhat protracted review of the work that I have here presented will make the subject clearer, and will serve to place more surely beyond cavil the fact that a gaseous or volatile compound of arsenic may be generated from decaying arsenical matter; hence the possibility of chronic poisoning from the presence of such a compound in the air of rooms papered with arsenical paper.

Of the experiments mentioned in the historical sketch, those of Schmidt and Bretschneider, Fleck, Hamberg, Selmi, Bischoff, Giglioli, and Kinnicutt are the most important. The others need not be considered, either because there was little or no chance in them for decomposition by mould, or on account of imperfect methods of experimentation or analysis. In the cases of Hamberg and Bischoff there was undoubtedly a volatile compound formed, though Hamberg proved it and Bischoff did not. Fleck and Selmi do not show conclusively that it was present.

The adverse results of the other four investigators, as well as those of my first series, are very easily explained. There was either too much arsenic for the arsenio-bacteria to tolerate, or else the latter were not present. It is worthy of note that, with the exception of Hamberg's experiments, whenever the unsterilized matter was allowed to decompose in a closed vessel, no arsenical compound was evolved, while exposure to spontaneous inoculation in the air developed an odor. This would seem to point to a tolerance when fresh germs can gain access to the material. In my own case I am inclined to think that absence of the specific bacteria was the chief reason for failure, though in many cases I used large amounts of arsenic. As to my experiments on the air of rooms, it is quite possible to explain the negative results by the choice of reagents and the amount of air aspirated. We have, however, sufficient evidence from Hamberg on this point.

The conditions necessary for the action of the moulds, especially the amount of arsenic and the quality of the culture ground, explain why the action has not been earlier discovered. Though it is not certain but that many moulds decompose arsenical matter, yet the intense action is thus far confined to four, *Penicillium brevicaule, Mucor mucedo, Aspergillum glaucum*, and *Aspergillum virens*. Further research may succeed in finding others. It is worthy of note that the most active mould is that which was discovered on decaying paper, and it must not be forgotten that a small amount of arsenic in a wall paper may be quite as good a source of the volatile compound as a very large amount.

I have not made any investigation into the nature of the compound, as such work would be trespassing on Gosio's field. I think the chances are that no arseniuretted hydrogen is formed, but that we have to deal solely with an organic compound of arsenic. This may perhaps form a "molecular" compound with the argentic nitrate, which, when the latter is acted on by hydrochloric acid or an alkali is set free. The odor of the solution was noticed by Hamberg, Gosio, and myself. Hamberg does not say whether it appeared after adding hydrochloric acid or in neutral solution. Gosio obtained it on adding alkali, and did not apparently detect it in the acid solution, while I discovered it in the desilverizing. Hamberg's yellow deposit may be identical with that noticed by Gosio and me, although Gosio does not agree with us in the finding of arsenic.

Hamberg and Gosio infer that the dilute argentic nitrate solutions absorbed only a part of the compound, and my experiments confirm this inference. Potassic permanganate probably oxidizes it completely. The non-absorption of the silver solution is an argument against the compound being arseniuretted hydrogen, for I have satisfied myself by experiment that a 2% argentic nitrate solution absorbs that gas completely when in small amounts, even when the gas is very greatly diluted with air.*

The use of argentic nitrate may partially account for some of the negative results of former investigators. With such an absorbent and the use of the simple Marsh test a comparatively large amount of the compound might have escaped notice.

* Although the argentic nitrate absorbs the arseniuretted hydrogen completely, yet when small quantities of arsenic are used, the amount recovered from the solution is rarely over 50% of the amount taken. Some rather odd results were obtained in investigating this question, the consideration of which is reserved for another paper.

In considering the nature of the compound, the only definite facts obtained are that it is formed in presence of oxygen, that the development is best from carbohydrates, and that arsenious or arsenic acid and their salts are best suited to the development. The only conclusion that may be drawn is that the volatile compound is an organic derivative of arsenious or arsenic acids. Yet the properties are such that it has little resemblance to any of these derivatives thus far isolated. Much work remains to be done before the composition can be determined, and Gosio is continuing his research to that end.

In this connection the researches of Selmi * are interesting. From a corpse, in which the presence of arsenic was established, he isolated a ptomaine in small quantity, but the test for arsenic in it was negative. In the stomach of a hog,† saturated with an arsenic solution and left to decompose, he was able to isolate two bodies containing arsenic. The first was obtained by distillation with steam, and had an intensely poisonous action like strychnine. The second was found in the residue from distillation, and the action of this resembled that of the ptomaines. In the urine of a dog ‡ poisoned by arsenic Selmi finds a volatile arsenic compound with tetanizing action, and considers it identical with the first of the two previously described. In no case were these compounds found in quantity sufficient to make an attempt at determining their composition. Husemann,§ referring to Selmi's work, thinks that an arsenical ptomaine could be generated from arsenical paper and paste, yet the properties of the volatile compound and the fact that it is best formed in non-nitrogenous ground seem to be against the formation of a ptomaine from these materials.

WASHINGTON UNIVERSITY CHEMICAL LABORATORY,
Saint Louis, September, 1893.

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* Atti della R. Accad. dei Lincei, [3], II., June 2, 1878.
† Mem. d. Accad. d. Scienze, Bologna, [4], I. 299.
‡ Ibid., [4], II. 3.
§ Arch. d. Pharm., CCXIX. 415.